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Rabbit Liver Metallothionein
Tentative Amino Acid Sequence of Metallothionein-B*

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The primary structure of equine renal metallothionein-1B was first reported by Kojima *et al.* Most recently, Kissling and Kägi have elucidated the amino acid sequence of human hepatic metallothionein-II and compared it to the known structure of the former. Huang *et al.* have also demonstrated the complete sequence of hepatic metallothionein-I obtained from the liver of mouse administered with cadmium. We report the amino acid sequence of liver metallothionein-B isolated from rabbits exposed to cadmium.

Rabbit liver metallothionein-A and -B were isolated from rabbits exposed to cadmium (by 21 subcutaneous injections of 1 mg/kg body weight with intervals of 2—3 days). On the second day after the last injection the animals were killed. To one volume of the liver homogenate in 0.02 M Tris-HCl buffer, pH 8.6, cold ethanol and chloroform (1.00: 1.05: 0.08:, by volume) were added with cooling. To the supernatant obtained by centrifugation three volumes of cold ethanol were added subsequently. The solution was allowed to stand at −20°C overnight. The produced precipitate was collected by centrifugation. It was dissolved in 0.02 M ammonium carbonate and then it was charged to a Sephadex G-75 column. The elution was carried out by use of 0.02 M ammonium carbonate. The Cd-binding protein fraction of low molecular weight on the gel filtration was further separated into the Cd-binding proteins, metallothionein-fA, a minor component, -A and -B, major components, by ion exchange chromatography on a DEAE Sephadex A-25 column.

Metallothionein was S-pyridylethylated with 4-vinylpyridine at pH 8.0 by using the modified method of Friedman *et al.* and S-^{14}C-carboxymethylated with iodoacetic acid-2-^{14}C at pH 8.0 by using the modified method of Crestfield *et al.* after the removal of metals by the treatment of 6 M guanidine HCl in 0.2 N HCl. Acid hydrolysates of the intact protein and the oxidized one with performic acid were analyzed by an amino acid analyzer. S-^{14}C-carboxymethylated metallothionein was digested with trypsin. The tryptic peptides were separated by ion exchange chromatography on a Dowex-50 (×2) column with gradient elution of pyridine-acetate buffer. Further purification of peptide was carried out by use of paper electrophoresis and/or paper chromatography. The fragment used for the automated sequence analysis was obtained by cyanogen bromide cleavage and by selective cleavage of labile bond between Asp–Pro with 70% formic acid. The fragments were

* *Metallothionein J.H.R. Kägi and M. Nordberg (eds) Birkhäuser Verlag, Basel/Boston/Stuttgart,* 1979, pp. 163~168.

sequenced by a JEOL JAS-47K sequence analyzer. Sequence analysis of peptide was carried out by the manual Edman degradation modified by Iwagana *et al.* PTH-amino acid was identified by gas chromatography, thin layer chromatography and back-hydrolysis to amino acid.

The gel filtration pattern of the precipitate with ethanol from liver homogenate of Cd-exposed rabbits and the ion exchange chromatographic elution profile of the Cd-binding protein fraction separated by the gel filtration were obtained. It was very convenient to use the ammonium carbonate solution in the separation system because the desalted preparation could be obtained only by lyophilization. Ammonium carbonate as well as Tris-HCl was effective in the separation of protein. Metallothionein-fA, -A and -B, prepared from the livers of two Cd-exposed rabbits, weighed about 50, 210 and 230 mg, respectively.

Rabbit liver metallothionein as well as mouse liver metallothionein contains 61 amino acid residues and no detectable amounts of histidine, tyrosine and phenylalanine. The total number of amino acid residues of rabbit liver metallothionein is identical with that of the other metallothionein reported. If the oxidized protein was hydrolyzed without removal of metals by the gel filtration on Sephadex G-25, the cysteinyl residues could not be completely recovered as cysteic acid. As the cysteine derived from the hydrolysate of the intact protein was eluted near the position of proline on the ion exchange chromatography for amino acid analysis, the numbers of proline obtained from the hydrolysate of the intact protein might be influenced with it. Neither amino acid composition of rabbit liver metallothionein-A nor -B may be therefore identical with those reported by Nordberg *et al.*

The tentative structure of rabbit liver metallothionein-B, presenting a single band on acrylamide gel electrophoresis, has been deduced from the results of the automated sequence analysis [Asp-2 or Pro-3 to Gly-11] and the manual sequence analysis of tryptic peptides in comparison with the known amino acid sequence of several metallothioneins. The estimated structure of rabbit liver metallothionein-B is indicated in Fig. 1. The sequence of Ser-12 to Lys-20 has not been confirmed. The substitution of Ala to Glx at the position of 23 may be proposed because the peptide of Cys–Lys–Glx–Cys–Lys in a small quantity was found in the tryptic digest. It may be related to the microheterogeneity of metallo-

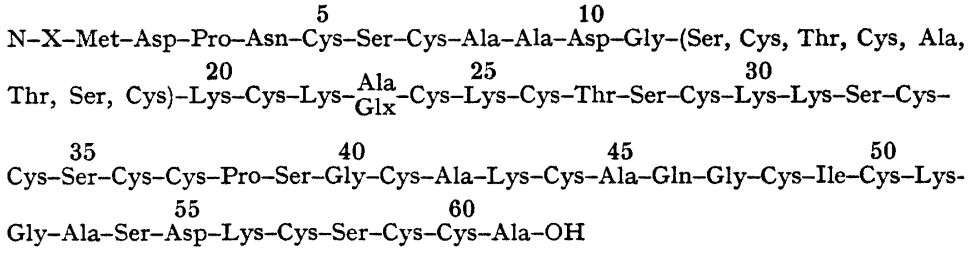

Fig. 1. Tentative chemical structure of rabbit liver metallothionein-B

thionein molecules, as pointed out in the other report, described by Kimura *et al.*. However, the chemical structure of metallothionein obtained from the animals exposed to cadmium is as a whole similar to that from the intact animal and human being, as illustrated by the present report and the other papers described by Huang *et al.*